

ATRX Directs Binding of PRC2 to Xist RNA and Polycomb Targets

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In characterizing ATRX as a regulator of X inactivation, we used two shRNAs to knockdown ATRX and then visualized the effects on Xist localization using FISH. In Figure 1C, we erroneously presented duplicate images of cells treated with shATRX2 showing both Xist and Xist plus DAPI staining in place of images showing cells treated with shATRX1. The quantification of Xist levels presented in Figure 1C reflects a separate qRT-PCR analysis and is not affected. The corrected figure, showing shATRX1-treated cells from the same experiment, appears below. Additionally, in the interest of experimental transparency, the accompanying figure legend has been updated to include the data acquisition parameters used in the experiment from which the images were derived. The figure and figure legend have been corrected online.

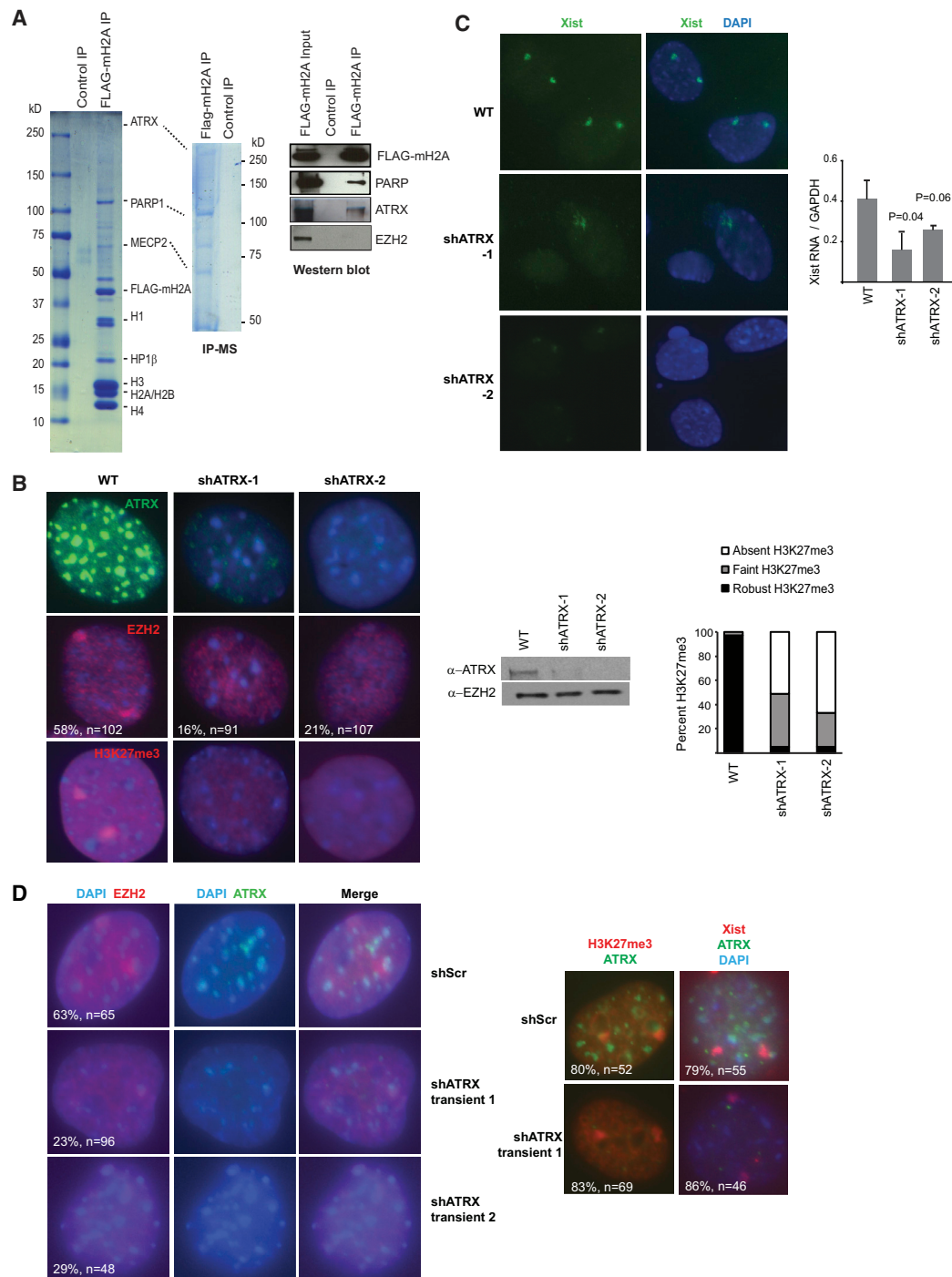


Figure 1. A Proteomics Screen Identifies ATRX as a Candidate XCI Regulator

(A) IP-MS: Colloidal blue staining of FLAG IP from control (293F) and FLAG-mH2A-expressing 293 run on a 4%–20% (left) and a 6% (right) SDS gradient gel. FLAG IP was validated by western blot.

(B) Left: Immunostaining of ATRX, EZH2, and H3K27me3 in WT and two independent stable ATRX-KD MEF lines (shATRX-1, -2). Sample size (n) and %EZH2 association with Xi are shown. Middle: western blot showing ATRX depletion but constant EZH2 levels in shATRX-1 and shATRX-2 female MEFs. Right: Patterns of H3K27me3 observed. n = 100–150 per experiment.

(C) Left: Xist RNA FISH in indicated fibroblast lines with FITC acquisition times of 500 ms and a gain of 61 for all samples. Right: qRT-PCR analyses of Xist RNA levels. SE bars from three independent experiments are shown with Student's t test p values.

(D) Left: Immunostaining of ATRX and EZH2 in MEFs transiently transfected with scrambled shRNA (shScr) and two shATRX constructs (shATRX-1, shATRX-2). Right: H3K27me3 staining and Xist RNA FISH show no change in the intensity or foci number after transient ATRX KD.